

Tag No. _____

Impuse: continuation of pg 124 - 125

amplified linearized puc / xmr 1 using 2 different molar sets of primers

36

37

38

39

tried with Tag and Tag + DV

trialed Mg 1.5, 2.0, 2.5, 3.0 mM cycling 94° 30" 1
 (94° 30" 1) 30
 200 μg dNTP
 1.4 μM primer product = 1275 bp.
 1 μg enzyme - Tag
 25 μg template

prepared 10X of each: Tag 1 # 3 Tag + DV 1 # 3
 11 1 # 2 11 1 # 2

1/20	33.8	330 μl	1.5	2	2.5	3
ox buffer	50					
dNTP	10		1.5	2	2.5	3 mM
Mg	-		7.5	10	12.5	15
primer 1	20		42.5	40	37.5	35
2	20					
Template	10		50			
enzyme	2	- 10 μl Tag + DV				

4.50
 45 μl / RX added 5 μl of Mg chf conc.

To Page No. _____

Assed & Understood by me,

Date

Invented by

Date

S. Steamer

12/8/94

Recorded by

12/9/94

S. Steamer

Project No. _____

Book No. _____

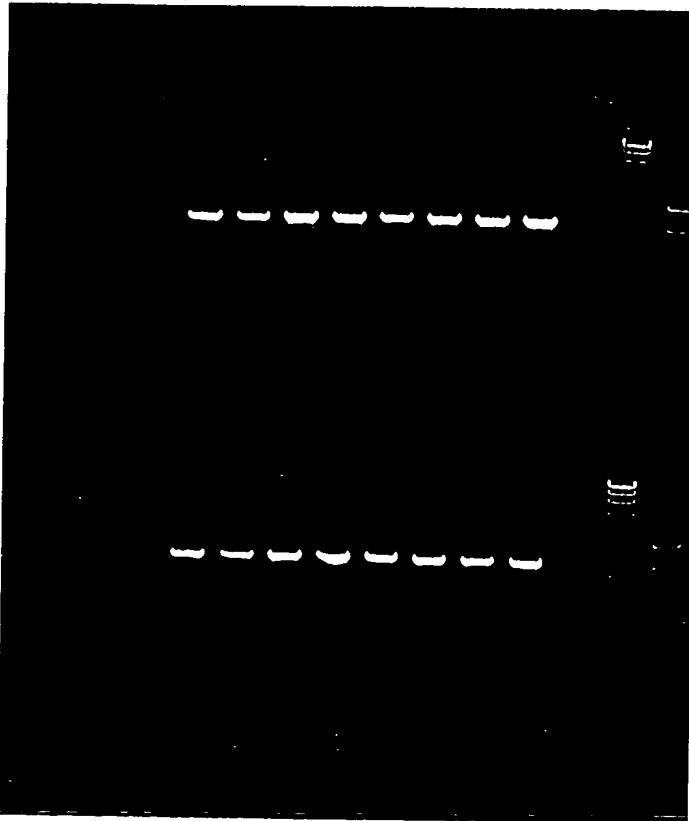
TITLE _____

128 T. 128

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Tag

0 1.5 2 2.5 3 my Mg



Tag + DV

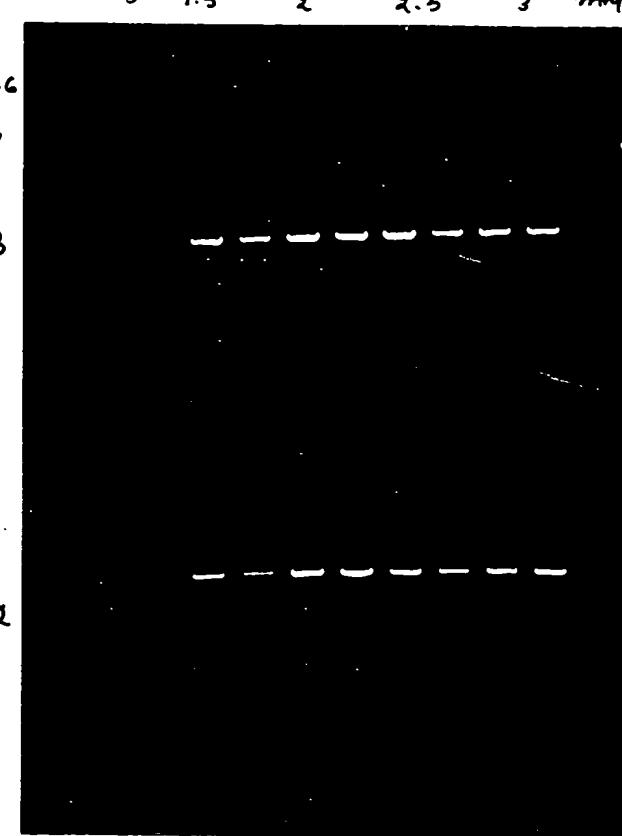
0 1.5 2 2.5 3 my

2936
v
37

x3

x2

2936x39



~ 1275 bp product.

Both primers set work with Tag as well as Tag + DV
 A bit of mispriming still - has to be gel purified
 great range of Mg tolerance.

pool (1) Tag 1.5 my Rx Separately) with x 3
 (3) 2.0 set of primer

(3) Tag + DV 1.5
 (2) 2.0

and phenol in
 ethanol p/

T Page

Witnessed & Understood by me,

Date

12/19/84

Invented by

Recorded by

R. Sitaranam

Date

12/18/84

Page No. _____

Added Ax from two tubes (duplicate & same) together is 30 μ l & made up the volume to 100 μ l 30 μ l

added equal amount of phenol: chloroform: 2x amyl alcohol
removed the aqueous phase after a spin of 5'.
phenol extracted again.

added 0.5 volume of 7M ammonium acetate and 2 ml of ethanol, added also a ml of Dextran T 500.
left at -20° , 1.5 hr

Spin down, remove ethanol, washed the pellet with 70% ethanol, spin down, removed the sup.

spin again to remove the residual ethanol
pellet visible, vacuum dried 5'

suspended in 17 ml $\text{of } 70\%$ removed 2 ml for gel (1) (2) (3) (4)

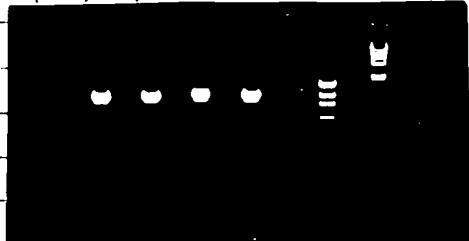
for 15 ml added

10.5 " H₂O

3.0 " 10 x buffer

1.0 " Afc ii (70/x)

0.5 " Aaf ii (240/x)



3.0 ml incubated at 37° 2 hr.

phenol extracted product seems to be around $\sim 150 - 200$ mg / hr
 ~ 45 mg / hr

To Page No. _____

Isd & Understood by me,

Date

Invented by

Date

14/1/84

Recorded by

12/5/84

R. S. Kaneman

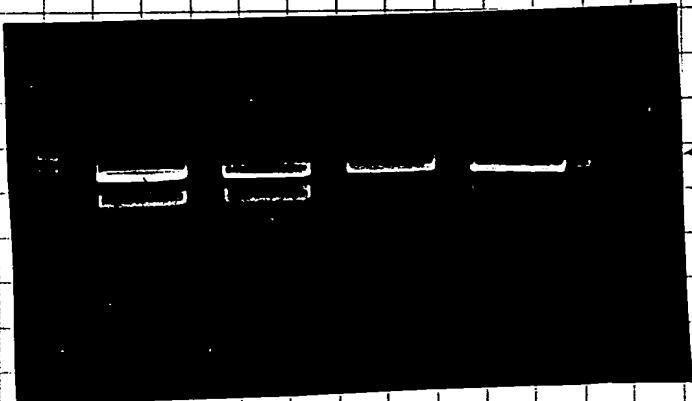
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15 μ l of leaf discs phenol chloroform extracted + ethanol precipitated + more cut with Pst 1 and Pst 3 in NEB buffer 4 for 2 hrs at 37°.

Ran on 1% gel and transferred to DAG paper and eluted the fragment in high salt buffer, over the 1M NaCl, 0.1M Tris pH 8.0, 5 mM EDTA

spun down in ethanol buffer added 50 μ l more P-
& centrifuged, pellet the ethanol, ethanol added in ~150 μ l → 500 μ l in the presence of 1 μ l of desalting T-400.

left at 70° 2 1/2 hrs. resuspended in 15 μ l of the ethanol wash, in 70°.



extracted to serve as insert.

$$\text{loaded on } 75 \text{ ng} \times 15 \mu\text{l} = 1125 \text{ ng (1275 bp)} \\ - 772 \text{ ng (875 bp)}$$

$$\approx 50\% \text{ recovery} = \approx 386 \text{ ng / 15 } \mu\text{l} \\ = \approx 25 \text{ ng / } \mu\text{l}$$

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Date _____

Revised by _____